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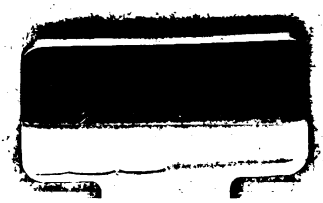

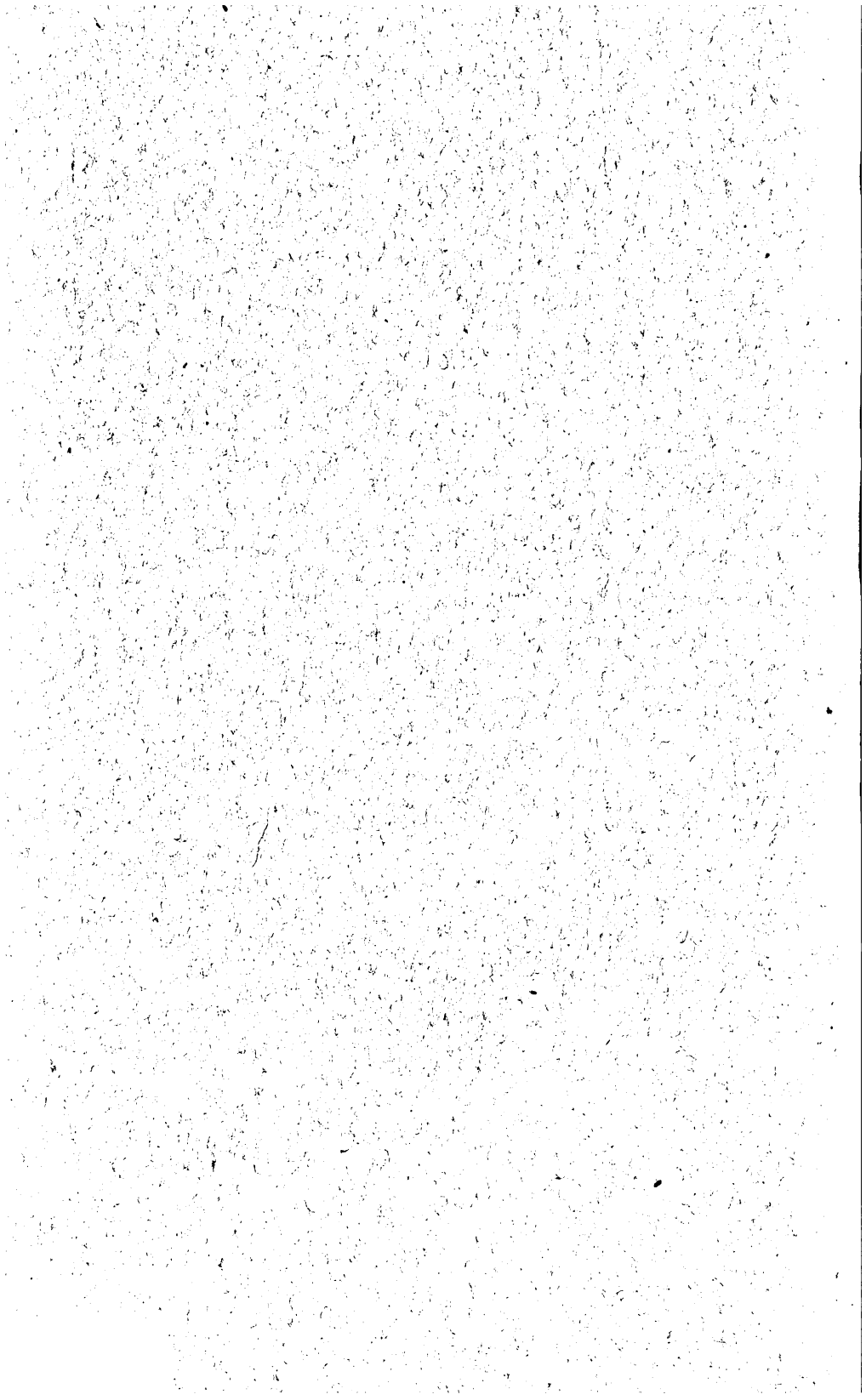

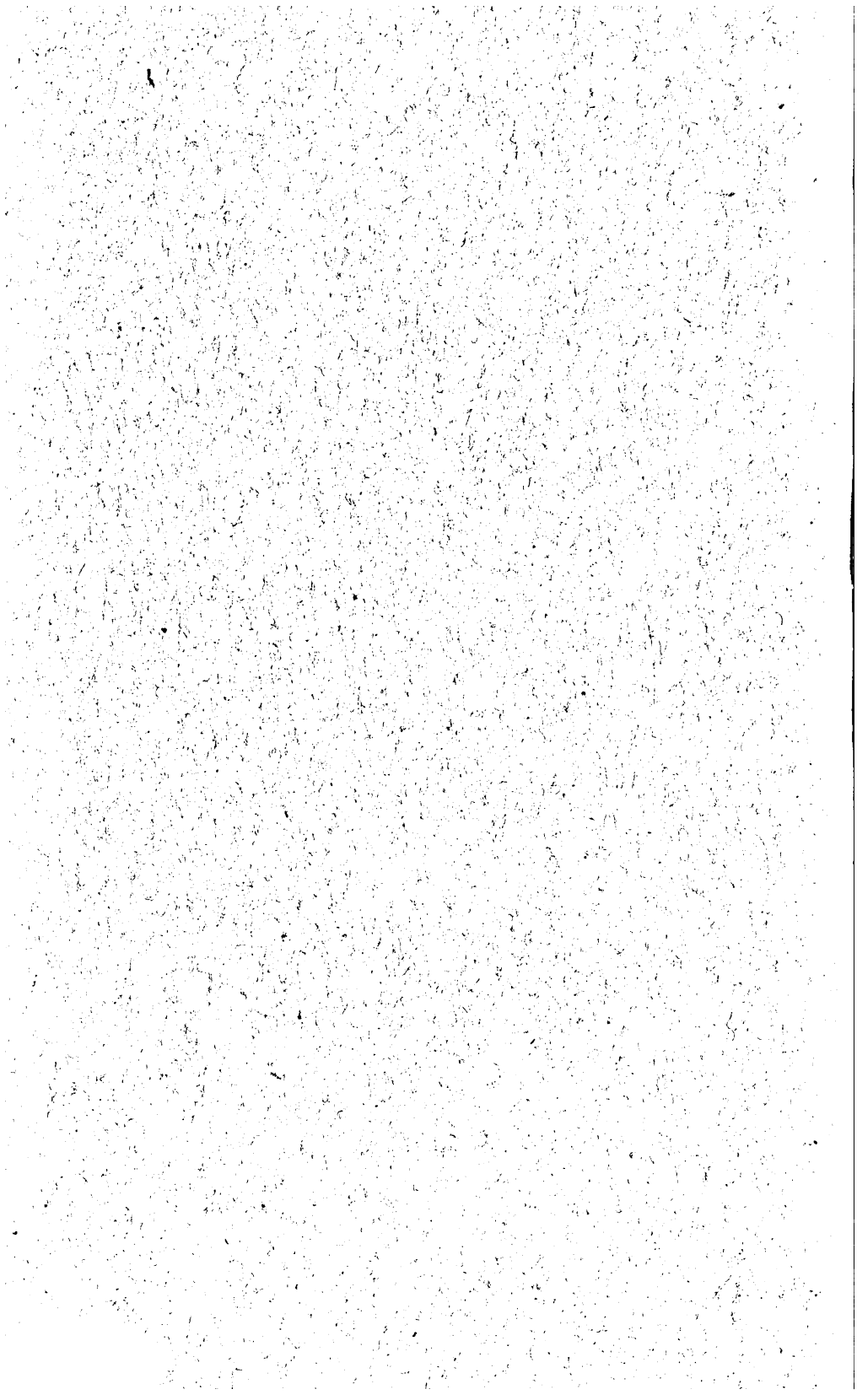

THE GROWTH AND ORGANIZATION OF THE STARCH GRAIN

BY

ROLLIN HENRY DENNISTON

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THE GROWTH AND ORGANIZATION OF THE STARCH GRAIN.

R. H. DENNISTON.

(With Plates XXXVIII–XL.)

INTRODUCTION.

Perhaps no subject was more studied by the earlier investigators of the plant cell than the starch grains and the plastids with which they are associated, and some of the first data which were established as to the organization of the cell were worked out in this connection. In more recent years the nucleus and its functions have claimed an excessive share of the attention of cytologists, and the recent summaries of our knowledge of starch and plastids show little advance beyond the discoveries of Schimper and Schmitz. Visible stages in the process of starch formation are still unknown, with the exception of one or two discoveries to be mentioned later.

The main steps by which our present views of starch and plastids were developed may be briefly summarized as follows: Our real knowledge of starch formation and the function of the chlorophyl bodies dates from the work of Sachs in 1862 (31, p. 365). In this paper Sachs advanced the doctrine that the starch in the chlorophyl grains is the first visible product of assimilation, a doctrine which has stood to the present time for the chromatophore without pyrenoids. This is the current statement of the textbooks. Timberlake, however (39, p. 624), has found that in *Hydrodictyon* the starch grains are formed from segments of the pyrenoids, so that in this case the starch is not the first visible product of assimilation.

Various theories were held by the earlier writers as to the nature and manner of origin of the chloroplasts or chlorophyl-bearing bodies of the cell. Mulder believed that they form from starch grains. Von Mohl thought that they have their origin in the cytoplasm. Schimper (34, p. 6) first developed the conception that the chloroplasts are permanent cell organs and arise only by the division of pre-existing similar bodies. He studied especially *Hyacinthus, Daphne* and *Torenia,* three plants widely separated systematically, and found chromatophores in the embryo-sac and egg cell of each. He also found chromatophores in the egg cells of the moss *Atrichum* and of the liverwort *Anthoceros.* On these observations he bases his doctrine that the chloroplasts are permanent cell organs and never arise *de novo* from the cytoplasm. Schimper also developed the doctrine that chlorophyl bodies, leucoplasts and chromoplasts are all homologous structures and proposed the term "plastid" to include them all (34, p. 30). He also believed that the leucoplasts and chloroplasts are capable of further metamorphoses into other sorts of plastids, but that the chromoplasts are fixed, as a rule, although he claims to have found the red and yellow chromoplasts in the carrot becoming green on exposure to light.

Schmitz (37) described the presence of chloroplasts in both the egg cells and spores of the Algae and agrees with Schimper that they are permanent cell structures for these plants. Von Mohl, in 1837, discovered that the chlorophyl grains can be separated into two substances, a green material soluble in alcohol and ether, and a colorless proteid which determines the form of the grain. Sprengel and Meyen believed the chlorophyl grains to be little vesicles. Nägeli was a supporter of the latter theory and thought that he could distinguish in the chloroplast a whitish membrane with green contents.

In more recent times the vesicular theory has had but few supporters, and Von Mohl's theory as elaborated by Sachs is the one generally accepted. According to this latter view, the chlorophyl grain is composed of a ground mass of colloidal consistency which in its chemical composition is probably a proteid. In this the green substance is imbedded.

Pringsheim concluded that the proteid of the chlorophyl grain consists of a spongy reticulum, which he called the "stroma." This stroma is saturated with a green solution consisting of an oil, in which the cholorophyl is dissolved. Pringsheim's results have been supported by those of Schmitz and Meyer, who were of the opinion that the stroma consists, not of a homogeneous plasmatic body, but of a porous, spongy mass. Meyer has also found dark-colored grains imbedded in the stroma, which he calls "grana." Schimper concluded that the chloroplasts consist of a colorless stroma containing numerous vacuoles filled with the green chlorophyl in solution.

Nägeli, in 1846 (23, p. 143), was the first to observe the leucoplasts. He described them as vesicles filled with starch. Schimper, in 1880 (35, p. 881), described the leucoplasts as specialized colorless organs of the cytoplasm which he called "starch formers." In a later paper he gave them the name "leucoplasts." According to Schimper, the earliest mention of chromoplasts is by Unger in 1846. Von Mohl (20, p. 361), in 1851, mentions the yellow crescent-shaped color bodies found in the yellow leaves of *Strelitzia*. Schimper (34, p. 2), in 1885, found evidence that they are homologous with the chloroplasts and may arise from the same rudiments in the egg.

The leucoplast has been termed by many physiologists a cell organ. We also consider a plant cell from one of the higher plants as a unit of structure of an organ of the plant. The higher plants are made up of tissues and organs, and from this standpoint the cell is the unit which cannot be sub-divided into units comparable to cells. But the cell is also an organism, and we may properly speak of those parts of the cell which have a permanent existence and perform a special function as cell organs. These are not homologous, however, with the organs of the higher plants, nor is the organization of the cell directly comparable to that of a plant part. From this standpoint we may call plastids and vacuoles cell organs. Verworn (41, p. 58) has proposed to call them "organoids," but there seems to be slight justification for calling them *organ-like* bodies when they belong to a morphologically different class.

The plastid, in the formation of the starch grain, shows several points of resemblance to the plasma membrane in the formation of the cell wall. Both are plastic proteid bodies from which carbohydrates are formed. The carbohydrates in each case show a similar stratified structure, the starch grains being formed of laminae which are laid down upon the surface of the grain much as the cellulose layers are deposited in forming the cell wall. There is, however, probably no great significance to be attached to this similarity.

THE STRUCTURE OF THE STARCH GRAIN AS INDICATED BY ITS STAINING REACTIONS.

Fritzsche (11, p. 129), in 1834, in the case of the potato starch grain, first noted the presence of concentric layers which completely surround what he called a spherical space. This space is usually located at one end of the grain. He believed the appearance of light and dark layers is caused by the varying water content, and that the density of layers deposited by day is different from that deposited by night. The outside layer is of special density according to Fritzsche, due to its becoming infiltrated with a large amount of proteid substance.

Von Mohl (21, p. 45) believed as did Fritzsche, that starch grains consist of superimposed layers of varying density, but composed of the same substance. He found no cavity in the center in fresh grains, but noted that one is developed on drying. Nägeli (24, p. 18) also thought that the layers of the starch grain are due to differences in density as a result of varying water content. He believed that the layers are closed vesicles, and that they form, not one outside the other, but one inside the other.

Schimper (36, p. 192) believed that the young starch grains are composed of a homogeneous dense substance. The grains increase in size, and a weakly refractive region develops in the middle. This is the hilum. The formation of the hilum causes a reduction in the strain on the surrounding starch, with the result that a loose layer forms between two denser ones.

Strasburger (38, p. 147) considered the weakly refracting layers as limiting lines or adhesion surfaces between lamellar complexes. Meyer (18, p. 107), holding that the grains are composed of crystalline units, explained the light and dark layers by the assumption that where there are many and large pore canals between the trichites the layers are loose, and that where the trichites are more closely packed the layers are dense. He observed also that, when grains are dried at 20°C over sulphuric acid in a vacuum, scarcely a trace of lamination remains.

Salter (32, p. 6) found that the starch grains show alternate light and dark blue layers with Flemming's triple stain. He concluded, since the aniline dyes are so easily removed from the stained starch grains, that staining is merely a process of imbibition of the coloring matter between the particles of starch substance. The layers taking the dark violet stain he supposes to be loose and watery, the less refractive layers of the unstained grain.

Meyer (18, p. 149) stained with methyl violet and then applied a very dilute solution of calcium nitrate, with the result that a large part of the stain was precipitated as a granular mass in what he holds to be the loose layers. Neither Meyer nor Salter furnished satisfactory evidence as to whether it is the dark or light layers of the unstained grain which take the deepest color in staining.

Fischer (7, p. 81) carried these precipitation experiments somewhat further by using picric acid as a precipitant instead of calcium nitrate. He allowed a drop of the dilute aqueous stain to dry on the section, then added a few drops of picric acid solution and washed with water. He found that the following stains were not at all taken up by the grain: nigrosin, Hessian purple, diamond red, carmin, anilin blue, cyanin and Congo red. The following gave a uniform coloration of the starch substance: acid fuchsin, corallin, eosin, crocein, tropaeolin, Martin's yellow and haematoxylin. The following gave fine-grained precipitates in the watery zones: fushsin, safranin, methyl blue, methylen blue, indigo carmin, indulin, methyl violet and gentian violet; the latter in the form of large crys-

talline grains. Radial crystal needles were formed by Bismarck brown, chrysodin, malachite green, brilliant green and thionin. Fischer says that it was plainly to be seen that the precipitates were not in the denser substance of the grain, but in the watery zones. The periphery always remains unaffected.

This peripheral layer of the starch grain has already been described in a preliminary paper (5), and is discussed further below; its staining capacity, form and constancy seem to indicate that it is different in composition from the layers inside.

The following experiment gives strong evidence that the violet stain passes through the orange layer readily but is not absorbed by it. In microtome sections, as already described, the staining of a large eccentric *Canna* grain may be watched under the microscope by allowing a solution of gentian violet to run under the cover. The layers inside this peripheral layer begin to absorb the stain at once, but the outer layer is not at all affected. The violet stain passes through the outer layer without being fixed. Of the layers inside, some are stained a deep violet, others take up only a small amount of stain and appear pale violet in color. This phenomenon is quite inconsistent with the view held by Salter that the outer layer is merely denser starch.

Rarely, in large *Canna* starch grains, we find the peripheral orange-stained layer followed toward the inside by a narrow dark violet layer, broadest at the posterior end (see Pl. XXXIX, Fig. 36). Next toward the inside of the grain there is a layer which is stained in some cases orange and in others pale violet. This layer is fairly broad and is continuous around the hilum. The remaining layers of the grain, with the exception of those immediately surrounding the hilum, are incomplete.

Oftentimes in the same material, a dark crescent-shaped line appears in the middle of a broad orange peripheral layer and at the posterior end of the grain (see Fig. 38). The orange material between this line and the inner violet layers usually shows a faint violet color though still predominantly orange. This appears to be the beginning of a violet layer,

since, in a somewhat later stage, the whole of the region between the dark violet line and the violet starch layer takes the violet stain and becomes the outermost violet layer of the grain. The dark violet line in but few instances was seen to pass around the hilum, but as the sides of the grain are approached the line becomes narrower and finally disappears. As has been pointed out, the peripheral layer stained by orange is transformed in the growth of the grain into violet-staining layers. This may be due to a condensation of the carbohydrate material, brought about by the abstraction of water, or to a more deeply seated chemical change. It is possible that this orange-staining substance is already carbohydrate material, which has been brought inside the leucoplast and which is then transformed by the addition of water into starch and gains the capacity to fix the violet stain. This would seem to be a more natural assumption than that starch can show such a variable reaction to the same stains, as assumed by Salter.

In the development of the cell plate in root tips, the equatorial zone was found by Timberlake (40, p. 97) to become filled with a substance that stains strongly with the orange of the triple stain. This substance appears to be entirely homogeneous and with ruthenium red or iron haematoxylin appears colorless while the cell wall is stained. Timberlake says: "The similarity of this substance to that of the cell wall, together with its presence in the region of the spindle in which the cell wall appears later, I have taken to signify the presence of a carbohydrate substance destined for the formation of the new cell wall."

In the germinating seeds of *Coix lacryma,* the walls of the endosperm cells disappear after the young plant has attained some size, and are apparently used to nourish the growing plant. These walls, while in process of solution, take the orange stain when the triple stain is used.

Preparations which I have studied show that the cellulin bodies in the cells of *Saprolegnia,* which are carbohydrate in nature, take a bright orange when stained by the triple stain. Noll (27) has shown that these bodies in the Siphoneae are

used to plug up holes in the walls of living cells, caused by wounds.

Thus, in a number of cases, we have a transition substance either in the formation or solution of carbohydrates, which shows a strong affinity for the orange stain. In the case of the starch grain and in the cell wall this substance appears to be a stage in the formation of still more complex compounds.

We find the very young starch grains either staining entirely orange or showing a large proportion of orange. We find an outer layer of orange material in the older grains, and by following the course of development of the starch grain, we are led to believe that the orange-staining substance in young and old grains is identical.

Newcombe (26, p. 49) has shown that the enzyme which dissolves starch in a number of plants is likewise able to dissolve cell membranes. As mentioned above, the cell walls of the endosperm of a germinating seed of *Coix lacryma* take a bright orange while in process of solution, probably by an enzyme action. In these cells the starch grains are also much corroded and show orange-stained borders of the corrosive channels.

It is quite possible that the substance first formed from starch by the action of diastase is the same that is present in the orange layer in the formation of the grain. The evidence certainly favors the view that the orange layer is a viscid mother substance, similar to that assumed by Mikosch, which becomes more and more concentrated by additions from without, until layers of starch form in its interior which first become visible in the dark blue line above referred to.

In the young grains, starch is deposited equally all round, but soon the grain shows an eccentric growth, the mother substance being formed more abundantly at one end. The plastid, however, continues its function of transferring carbohydrate material to the mother substance inside, which is too viscid to allow the additions from the thicker part to diffuse readily to the mother substance at the opposite end of the grain, under the thinner part of the plastid. In this manner, the mother substance under the thicker part of the plastid soon becomes

saturated, and it is on this side that the thick portions of the starch layers are deposited. This assumption is in harmony with the fact that, when the eccentric layers begin to form, they are simply thinner on the anterior end, then they become incomplete, and finally are laid down on the posterior end only.

The question as to the existence of a specially defined outer layer of the starch grain was early raised and has been discussed by various authors. Fritzsche (11, p. 138), in 1834, was one of the first to point out that the peripheral part of certain varieties of starch grains shows a somewhat different reaction to stains than the central portions, and supposed it to be due to the presence of certain foreign matters in this layer which render it more resistant.

Nägeli (24, p. 186), in a paper published in 1847, held that the outer part of the starch grain is composed of cellulose. This, however, was soon disproved. Crüger (4, p. 41), in 1854, described a layer between the protoplasm and the starch grain which "does not stain with iodine, nor does it stain brown as readily as the surrounding protoplasm." As he makes no mention of the plastid in other connections, it is possible that this is what he saw, and his figures bear out this view.

In 1885, Mikosch suggested the existence of an intermediate region between the grain and the plastid, which is filled with the so-called "mother substance" for the grain. Mikosch's conception agrees well with what I have described below as the specially differentiated peripheral layer. Meyer (18, p. 149) denies the existence of such a mother substance and says that normal starch grains do not possess a specially differentiated outer layer, but that he found such a layer in a few cases in starch from a potato.

Such a layer is described by Salter (32, p. 40), who believes that it is composed of starch but that it is denser than the remainder of the grain. This density is due, according to Salter, to the fact that the loose layers become much thinner at the periphery of the grain, hence the peripheral portion of the grain is made up chiefly of the dense layers which join and run around the hilum in large eccentric grains such as those

of *Canna* and potato. Salter's drawings in a number of cases show this peripheral portion of the grain stained bright orange, but he does not attribute this to a difference in composition between this and the inner blue-stained portion.

I have found a peripheral layer present in some cases and not in others, and have further undertaken to determine the conditions under which it occurs, as discussed below. The method of proving, by the use of Flemming's triple stain, that a differentiated peripheral layer is present in certain grains and perhaps at certain stages in the development of all starch grains, has been described in detail, but without figures, in a previous paper (5).

The method is in brief as follows: A series of slides was prepared by exposing for different lengths of time to the various stains. In every case, the slides were exposed to the safranin for five minutes; after washing in water, six slides were exposed to gentian violet for five minutes each, then treated with orange for the following different lengths of time: one minute, five minutes, ten minutes, twenty minutes, sixty minutes and three hours. It was found that with the exposure to orange for one minute, the peripheral layer is stained a pale violet. The inner layers are stained a dark violet. With the exposure to orange for five minutes, a peripheral orange layer is plainly differentiated, extending entirely around the violet portion of the grain. This orange-staining peripheral layer appears in all the other preparations in this series. Where the exposure to the orange is for sixty or one hundred minutes, the layers inside still show a pale violet color. Where the exposure to orange is for three hours, the grain becomes orange in color, except for a few layers midway between the hilum and the posterior end of the grain which remain violet.

It is seen from this series of slides that when once the grain is stained violet, a long exposure to orange is necessary to remove the violet from any of the grain but the layer in question, while but a few minutes suffice to remove all traces of violet from this layer and to replace it by orange. This seems to indicate that differences either of a chemical or physical nature exist between the body of the starch grain and the outer layer.

I have studied most fully by this method the large eccentric grains of *Canna* and of potato, which show the orange layer most sharply if the starch from rhizomes of growing plants of *Canna* or from ordinary fairly grown potatoes is studied. In the *Canna* grains, the orange zone extends around the inner violet-stained layers as a complete layer, usually fairly uniform in thickness (Pl. XXXIX, Fig. 37), or sometimes somewhat broader at the posterior end of the grain (Pl. XXXVIII, Fig. 1). Sections cut from any given portion of the rhizome of *Canna* usually contain starch grains which show a certain uniformity in staining and differ slightly from those in other regions, but in most cases a peripheral orange layer is present on a large proportion of the grains, whether the sections are taken from regions nearer to or more remote from the growing point.

A rhizome of *Canna* which had lain dormant through the winter, but from which a strong shoot was growing at the time the preparation was made, showed, almost invariably, starch grains with orange-staining peripheral layers. The outer starch layers of these grains showed slight corrosion, and no doubt these grains were being used for the development of the shoot.

The small grains, which show their laminae distinctly, show this peripheral layer with great uniformity. In certain preparations, the large grains do not show an orange layer, while the smaller grains in the same preparation show the layer distinctly.

In other material it was impossible to demonstrate a differentiated peripheral layer, either on the large or small grains, and it seems fair to assume that these have been in a growing condition.

The starch grains in the stem of *Pellionia Daveauana* are large and of the eccentric type. In the outer part of the cortex, the grains are not so large and are enclosed in relatively large chloroplasts (Pl. XXXVIII, Figs. 17-20). The grains nearer the center of the stem are large, and the chloroplasts are extended as thin membranes somewhat thicker at the posterior side of the grain. When treated with the triple stain, a peripheral orange-stained layer is clearly differentiated. The

layers of starch in the body of the grain are violet in color and fairly uniform in shade.

In the parenchyma cells of the fleshy rootstocks of *Dieffenbachia seguina,* large starch grains are present. They are elongated in form, with the hilum close to one end. In many of these grains, the effect of a change in position of the plastid is shown. In these grains the laminae are in two series which are often at nearly right angles to each other (Fig. 32). When stained with the triple stain, an orange layer is differentiated at the periphery and the interior layers are stained violet.

The starch grains in the parenchyma of the false bulb of *Phajus grandiflorus* are large, and the hilum is situated near one end, often in a small projecting tip. The leucoplast in these grains is often distended by a linear crystal of calcium oxalate. A peripheral orange layer is differentiated by the triple stain.

The starch grains of wheat, barley and rye are lenticular in form with a central hilum. Both in material which has been fixed and in fresh material, an orange-staining peripheral layer may be demonstrated by the use of the triple stain.

The starch grains from *Zea mays* are polygonal or rounded in form, with a distinct central hilum and concentric layers. The leucoplast is demonstrated with difficulty, but an orange-staining peripheral layer is present on many of the grains.

In the endosperm of the seeds of *Coix lacryma jobi,* the starch grains have a polygonal form. The hilum is central, and the grains with but few exceptions show a broad orange-staining peripheral layer. In the germinating seeds of this plant, the cellulose cell walls are stained orange with the triple stain. It is probable that the cellulose of the cell wall is modified in some way to make it available for the use of the growing plant in germination. A similar orange-staining substance is produced in the formation of the cell walls, as shown by Timberlake.

Of seven different commercial starches prepared as chemically pure starch by Eli Lilly & Co., two showed the peripheral layer in nearly all the grains; these were potato and tapioca starch. In wheat, bean, corn and oat starches, peripheral lay-

ers were found in a few grains. The pea starch showed no differentially stained peripheral layer in any case. It is quite possible in the case of these chemically pure starches that the method of preparation might remove in some cases any peripheral transition layer.

A short treatment with iodine in aqueous solution, in the case of *Canna* starch, leaves the peripheral layer perfectly white, while the inner parts of the grain stain blue. If the iodine acts for some time, the peripheral layer gradually acquires a blue color. With iron haematoxylin, in starch grains of *Canna*, potato and wheat, this layer does not stain, while the rest of the grain is colored in each case. With Correns' silver nitrate precipitation method, these starches show no precipitate in this peripheral region. These differences in staining qualities certainly show that either chemical or physical differences exist between the body of the starch grain and this outer layer, and as this layer is present especially in young grains and in grains in the process of solution, it may be properly called a transition layer.

If the exact conditions and stage of growth from which the starch was taken could be ascertained in every case, the explanation of the presence or absence of a transition layer might be at once apparent. If the orange-staining peripheral layer is a transition substance, then we should expect to find it on starch grains from parts of plants which are not fully developed or where storage of starch is going on, such as growing tubers and rhizomes and unripe seeds and fruits, and an examination of the facts leads us to believe that such is the case. As noted above, in the rhizome of *Canna* the starch grains were probably still in an actively growing condition. The *Canna* rhizome was from a growing plant, and the starch grains were probably still immature.

The potato was one taken from the bin, and the condition of the plant at the time the tuber was gathered is, of course, unknown; but of a number of potatoes which were apparently mature, none were found in which the starch grains did not show the peripheral layer in the majority of cases. It may be

that in such watery tubers the peripheral layer never passes into typical starch.

In the cases of *Phajus*, *Dieffenbachia* and *Pellionia*, the plants from which the starch preparations were made were actively growing. In the commercial starches, the method of cleaning and preparing will certainly have much to do with the presence or absence of any peripheral portion of the grain, as well as the relative maturity of the parts of the plant from which the starch was taken.

In the case of pea starch, no peripheral layer could be demonstrated, and it is possible that the starch matures more rapidly in this plant than in the others studied. Bean starch showed but few grains with an outer differentiated layer. These were grains of small size and probably immature.

In preparations of *Canna* which show the starch grains to be partially dissolved by natural corrosion by diastase, an orange layer appears quite constantly at the periphery of the portion of the grain remaining. The width of this orange layer is usually quite uniform although but a fragment of the laminated grain may remain inside. The structure and appearance of these corroded grains will be more fully discussed below. The fact that we have orange-staining layers in grains in process of solution as well as in grains in process of formation throws further light on the nature of this material as a transition substance.

The appearance of the so-called strata or concentric layers of the starch grain as seen when mounted in water has been variously characterized by different authors. Strasburger describes the layers as appearing to contain varying amounts of water, and as separated by dark limiting lines. Meyer discusses the varying appearances obtained by focusing through the grain with low and high magnifications, but gets no new data as to the composition of the layers. Salter uses the terms "dense" and "lax" to describe the layers of the starch grain.

I have naturally found the median optical section of the grain the most favorable for study, and my descriptions are

based on the appearance of the strata as so seen. In this case they are of course approximately at right angles to the plane of the slide and appear sharply defined.

The starch grains in the rhizome of *Canna* have been found to be specially favorable for comparative studies of the unstained and the stained grains. The attempt has been successfully made to identify a layer or series of layers in an unstained grain and then in the same grain to determine successively the effect of different stains on these same layers. The material was fixed in Flemming's weaker solution and imbedded in paraffin. Microtome sections 10 μ in thickness were used. The sections were fixed to the slide and the paraffin removed by xylol, the xylol removed by absolute alcohol, and the sections were then mounted and examined in water.

I selected a large grain from a slide prepared in this manner. At the posterior end of the grain there appeared two broad, highly refractive layers (Pl. XL, Fig. 42 A, *a* and *b*). These are broadest in the middle line of the grain and thin out gradually to the sides. Between these layers there is a layer (*1*) which has the appearance of being an open water space. It is thickest at the median line and tapers gradually to the sides. Toward the hilum from *b* there is a dark, slightly refractive layer separated from *b* by a dark line. This layer appears to be divided, the inner portion being paler in color. The remaining layers of the grain appear but faintly, with the exception of those immediately surrounding the hilum, which are fairly distinct. The appearance of this grain as just described is that seen in median optical section. If the focus is raised or lowered we may get the appearance of light layers where there were dark layers and vice versa. This is due of course to the curvature of the layers, a change in focus bringing into view the next inner or outer layer which may be different in refractive index.

The grain above described, mounted in water, is magnified 980 diameters and drawn by the aid of a camera lucida. If now the grain is kept under observation and a dilute aqueous solution of iodine drawn under the cover by placing drops of the solution at one side and filter paper at the other, the grain

will slowly take on the characteristic blue color. In a short time the whole grain is colored uniformly a deep, dark blue, but it is noticed that certain layers take the blue color more readily than others. The outer part of the refractive layer *a* is the first to show the iodine reaction, and in this it is followed by the inner part of the same layer. These two parts of the layer *a* thus become clearly differentiated, and the inner part (*a"*, Fig. 42 B) takes the darker stain.

Layer *b* takes on the blue color but slowly and remains for some time the lightest layer in the grain. It shows a marked contrast to the two darker layers *a"* and *c*. Layer *c* stains readily and in a short time becomes the most deeply stained layer in the grain.

Although layers *a* and *b* are the first to show the effect of iodine, it appears to enter gradually around the peripheral portion of the grain until the whole grain is colored a dark blue. As a result of treating with iodine, the open region *1* disappears and between *a"*and *b* a dark line appears. There is also a dark line between *b* and *c*.

If we now wash out the iodine by drawing alcohol under the cover glass, we shall get the following results: When the washing out is partially complete, it is seen to have been removed first of all at the periphery of the grain. The grain is kept constantly under observation, a drop of 95 per cent alcohol being placed at one edge of the cover and drawn through by a piece of filter paper at the opposite side. The layer *a* of the unstained grain is now seen still more clearly to be composed of two layers, the outer one becomes colorless and the inner one a medium blue. The crack-like region *1*, which appeared as a dark line in the grain stained with iodine, still appears as a dark line. This region is probably filled with an aqueous solution in the unstained grain, and closes up when iodine is applied.

Layer *b* of the unstained grain, which colored but slightly in iodine, shows two regions when alcohol is applied, an outer pale blue region and an inner darker region. The dark line *2* which is present at the inner border of *b* in water and in iodine, does not change in appearance with the alcohol.

Layer *c*, which takes a dark blue color in iodine, becomes pale blue when alcohol is used. The washing out of the iodine by alcohol causes a slight shrinkage in the grain, most noticeable at the hilum. If now this same grain is kept under observation and stained by gentian violet followed by orange G, some further interesting data are obtained. Here again the stains are applied at one edge of the cover and drawn through by means of filter paper. The gentian violet is allowed to remain five minutes; it is then washed out by water, and orange G is applied and allowed to remain three minutes. This is washed out by absolute alcohol, and the preparation is cleared by clove oil. During the time of this process there is no change in the position of the grain, and a third drawing was made of the same portion of the grain showing the appearance of the strata when stained by the gentian violet and orange. (Fig. 42 C). Around the entire periphery of the grain there appears an orange layer (*a*). This corresponds approximately with the pale blue layer *a'* of the grain stained with iodine. The next layer *a''* is pale blue and corresponds with *a''*. Following this layer there is a thin pale blue layer. This is layer *1* of the grain treated with iodine. The pale blue layer *b* of the grain stained with iodine is split up into two layers, *b'*, a dark blue layer, and *b''*, paler in color, in the grain stained with gentian violet.

The dark line *2* appears as a narrow, very pale blue layer. The dark blue layer *c* appears in the same position as the dark blue layer *c* of the grain stained with iodine.

The results of this staining experiment are summarized in the following table:

Starch grain represented in Plate XL, Figure 42.

A (In water.)	*B* (In iodine solution.)	*C* (In gentian violet and orange G.)
a—refractive layer	*a'*—light blue *a''*—dark blue	*a'*—orange *a''*—light blue
1—crevice	*1*—dark line	*1*—narrow blue layer
b—refractive layer	*b*—pale blue layer	*b'*—dark blue layer *b''*—light blue layer
2—dark line	*2*—dark line	*2*—narrow light blue layer
c—dark, slightly refractive layer	*c*—dark blue layer	*c*—dark blue layer

We find thus that layers which appear single when mounted in water may be really double or perhaps even made up of a number of layers. The differentiation of the parts in such a case is not sufficient to enable one to make them optically distinguishable as individual layers when the grain is mounted in water. That, none the less, considerable difference exists between these parts is shown when the grain is stained.

The regions of the dark lines *1* and *2*, marking the surfaces of *a, b* and *c,* swell somewhat in the treatment with gentian violet and orange and stain a pale blue color. It is probable that these are thin spaces filled with watery solutions which are relatively slightly refractive.

When gentian violet and orange are used after iodine, the spaces apparently open slightly and seem to contain some starch which is stained a pale blue. It is further noteworthy that although the layer *c* is not so refractive as *b* in water, it stains fully as darkly as the darker portion of *b*. The view is thus on the whole confirmed that the cause of the difference in appearance of the layers in grains mounted in water is due to their difference in density which in turn results from differences in composition, the layers which contain the largest proportion of starch and the smallest proportion of water being the more highly refractive.

A second grain (Fig. 43) from the same *Canna* material was treated in the same manner as the grain just described. Drawings were made of identical portions of the outer layers of the grain as they appear with the different reagents: Fig. 43 *A* shows the grain mounted in water; *B* shows the grain treated with iodine, the drawing having been made before the grain was completely darkened by the reagent; *C* shows the iodine partly washed out by the alcohol; *D* shows the layers stained by gentian violet and orange. The following table explains the appearance of the layers when treated with the different reagents:

Starch grain represented in Plate XL, Figure 43.

A (in water.)	*B* (in iodine.)	*C* (in alcohol.)	*D* (in gentian violet and orange G.)
a—Highly refractive region.	*a'*—Layer which has not so fully taken on the nature of starch, hence is faintly blue in color. *a''* —A blue starch layer.	This layer is now pale blue in color, the color becoming lighter from inside toward periphery. There is no sharp line separating two parts.	The layer *a'* is of different composit'n and takes orange; *a* " is starch and takes gentian violet like rest of grain.
1—A dark line, probably a crack filled with watery colloidal mass.	This layer is broader and paler in color.	In alcohol this layer is still broader.	This layer is about the same width as in alcohol. It stains pale blue.
b—Highly refractive layer.	Contracted slightly and stains blue.	Contracted a little more and blue partly removed.	Characteristic blue stain with gentian violet.
2—A dark line similar to *1*.	*c* and layers anterior to *c* have contracted, leaving space at *2*.	Contraction goes on with consequent broadening of *2*.	Stains pale blue, contains relatively small amount of starch.
c—Slightly refract'e layer.	In iodine this layer stains uniformly with those next to it on inside. It is pale blue in color.	The iodine is easily removed, leaving layer pale blue in color.	This layer stains less deeply than *a* or *b*.

In this grain there is an outer refractive region which has the appearance of a single layer when the grain is mounted in water. This outer region is similar to the region *a* in the grain represented in Figure 42. In both grains it consists of two layers which are differentiated by iodine or by gentian violet and orange. When iodine is washed out of this region, it is removed from *a'* first and then gradually from the outer part of *a''*, so that the sharp line separating the two layers disappears.

The dark line *1* which separates *a''* from *b* becomes broader in iodine, and is no longer dark, but swells somewhat and stains a pale blue. It becomes broader by the contraction of the layers *a'* and *b* and stains less deeply, probably because it contains less starch than these layers. This layer stains less intensely with gentian violet than *a''* and *b*, which bears out the assumption that it contains less starch.

Layer *b* shows the same reactions to stains as *a''*. The dark line *2* shows the same characteristics as *1*. It does not

appear as a dark line except in water, and in its place there appears a layer which is pale blue in iodine and in gentian violet and which probably contains very little starch material. This layer appears to swell when iodine or alcohol is applied, due no doubt to the contraction of adjacent layers.

Layer *c* is less refractive in water than either *a* or *b* and probably contains less starch material. In iodine and also in gentian violet it contracts considerably and takes a pale color with both these reagents.

From the study of grains such as the above it is plain that the ordinary conception, that the visible elements of the grain consist of denser layers of starch alternating with more watery layers, must be extended to include the appearance of sharp lines marking the boundaries of the highly refractive layers and also spaces which are practically open crevices between the layers and which may become wider or narrower with the contraction or expansion of the denser layers or of the entire grain.

The grain of Figure 42 shows the existence of crevices most clearly. Such a crevice appears conspicuously between *a* and *b* when the grain is mounted in water. It closes up and appears as a dark line when the grain is mounted in iodine solution, and as a narrow light blue layer in gentian violet. Similar crevices exist at *2* in the same grain and at *1* and *2* in the grain of Figure 43, although the latter are narrower. Such crevices are most sharply distinguished from the starch layers by the readiness with which they change their width on the application of reagents to the grain. Their width is apparently entirely determined by the swelling power of the adjacent layers, and it is to be noted that the inner layers of the grain appear to be less dense than the posterior layers; they contract noticeably when alcohol or iodine is applied.

The refractive layers also vary in thickness and density. The thicker layers do not all color with the same intensity with iodine or with gentian violet. It is also clear that those layers which take the deepest color are in general the densest layers.

They are highly refractive in water, and the stains are removed from them with greater difficulty than from the layers which are not so dense.

ANALYSIS OF STRUCTURE BY CORROSION AND SOLUTION.

Blocks of starch-bearing tissues were taken from a rhizome of *Canna* in a region which had recently produced a vigorous shoot. The material was fixed, imbedded and sectioned in the ordinary way and Flemming's triple stain was used.

Many of the large eccentric grains were found still enclosed by plastids and invariably showed corrosion. Where the plastid is thickest the corrosion of the grain appears the greatest in extent, and frequently the broader posterior end of the grain beneath the thicker part of the plastid is reduced to a mere point (Fig. 39). Occasionally grains appear in which solution has taken place at both ends more strongly than in the middle. In such cases a spindle-shaped grain results. Frequently the anterior end is reduced to a point and takes the orange stain (Fig. 30).

In these large eccentric grains from *Canna*, the corrosion in the plastid seems to be upon the surface of the grain only, and all the layers of the grain which reach the surface seem to suffer from the action of the diastase in nearly the same degree. A slight difference in the rapidity of solution in certain layers is noticed, however, in some preparations. The pale violet layers are acted upon with the greatest rapidity. The ends of the dark violet layers project farther on the corroded margin than do the ends of the light layers (Fig. 39).

The appearance of these corrosion channels in the wheat starch is similar to that of the figures which Goldschmidt has described as forming in $CaCO_3$ spheres when treated with HCl, as will be noted further below.

Starch grains artificially corroded by solutions of diastase are more favorable material for making observations on the behavior of the different layers of grain. If slides with sections of starch-bearing tissues are placed in a tube of diastase solution, to which a few drops of chloroform are added to prevent bacterial growths, the corrosion usually takes place in two or

three days if the temperature is kept at about 40°C. The principal difficulty in the use of this method lies in the loss of sections in the diastase, but usually a sufficient number remain to give a few corrosion figures.

The large eccentric grains of *Canna* (Pl. XXXVIII, Fig. 28), treated in this manner for two days, show a strongly marked peripheral orange layer except for one or two small areas, usually on the posterior end. The conspicuous outer dense layers have been dissolved in a number of spots, but remain fairly intact. Considerable substance has been removed from the interior of the grain, and parts remaining in the interior of the grain quite generally take the orange stain (Figs. 26, 27). In some cases, the inner portions of the *Canna* grain have been completely dissolved and there remains only a shell made up of parts of the outer violet layers (Fig. 29).

The highly refractive layers in the unstained grain contain, as we have seen, a relatively small amount of water, and these are the layers which would naturally be expected to be most resistant to the action of diastase. We have found that in some cases the refractive layers of the unstained grain are not homogeneous but consist of a number of layers. Some of these component layers take a deeper and some a paler color. Evidence of the same condition is found in corroded grains, and in every case the parts which are stained most deeply with gentian violet are least acted upon by the diastase.

In oval starch grains from germinating wheat and barley which are enclosed by the plastid and which show the effect of diastase action, the corrosion does not take place evenly around the periphery, but peculiar pits and canals are formed beginning at the periphery and extending irregularly into the center of the grain.

In the later stages of corrosion there seems to be a tendency on the part of the corrosion channels to follow concentric lines in the interior of the grain. An effort was made to learn which layers were most attacked but without success. The interior of the grain takes an almost uniform stain after diastase action has gone on for some time. In the earlier stages of diastase action, however, in certain wheat grains in which the

corrosion channels have penetrated but a few layers, there appear irregularities along the walls of the channels; but no good evidence could be secured as to whether the light or the dark layers were more susceptible to diastase action.

A noticeable fact in connection with these corroded grains is that the portions of the layers bordering directly on the corrosion channels show a margin of orange-stained material which blends gradually into the violet of the unaffected portions.

Krabbe's observations led him to believe that the substance of the starch grain is removed, molecule by molecule, and that there is no general penetration of the grain. He used iodine as a stain and found that the parts of the grain remaining showed no difference in staining properties from the intact grain. His results, when compared with the conditions observed in Figure 28, show how little reliance can be placed on observations of corroded grains in water, even when stained with iodine.

Flemming's triple stain shows the borders of the corrosion channels plainly differentiated in corroded *Canna* grains; the material bordering the canal takes the orange stain. Iodine is not a good differential stain, and does not show slight differences either in the composition or the structure of the starch grains. Whether or not there is a penetration of diastase in all cases beneath the surface of the corrosion canals, there is plainly, in the case of *Canna* starch, a transition layer in all surfaces which are being corroded. The presence of orange-stained material over the entire corrosion surfaces of the grains suggests very strongly that a substance is found at the time of solution of the grain similar to that present on the surface of grains which are being formed.

The experiment was tried of crushing large *Canna* grains which had previously been stained by the triple stain. The crushing was effected by pressing on the cover of a freshly made slide with an eraser before the balsam had hardened. A number of deep radial cracks running from the surface inward are formed in this way. This fact probably has no significance in determining the finer structure of the starch

grain, as substances which we know are not formed of radially placed elements show radial cracks when crushed in this way.

The experiment was next tried of crushing the unstained grains and then staining with gentian violet and orange; it was found that much of the inner portion of the grain takes the orange stain, only a few layers at the outside taking the violet.

The natural inference drawn from the result of the above experiments is that the orange does not stain the inner parts of the intact starch grain because it does not reach them, but on the other hand it must not be supposed that the orange layer at the outside of the grain is simply the effect of the washing in of the orange. On some of the crushed grains which stain orange in the interior there is a peripheral orange layer, and on others there is not. It is to be remembered, also, that the width of the orange layer does not vary proportionally to the length of time through which the orange is allowed to act on the grain. If the grain is stained for five minutes each in violet and orange, the orange peripheral layer is differentiated when present, and there is no essential change in appearance though the time of the exposure to orange be doubled.

The appearance of the orange-staining material in the interior of the grain seems to indicate that this portion of the grain is also somewhat different in composition from the peripheral violet layers, and it is quite probable that we have in the interior of mature starch grains a change taking place which results at the same time in a loss of laminated structure. The exact nature of this change is difficult to determine experimentally, but it may well be a change from a less to a more soluble condition; if we can accept Meyer's conclusion that the starch grain is made up of amylose and amylodextrine, it is possible that this portion of the grain contains a larger proportion of the more soluble amylodextrine. Fischer (10) has found that this portion of the grain is in a semi-fluid condition in grains soaked in water.

In a preparation made from a grain of wheat which has begun to germinate, the starch grains are more or less corroded. Often the starch grains will be corroded in such a way that they will appear as if cut off on one or more edges (Figs. 40,

41), and on these corroded surfaces there appears an orange-staining area in which no lamination whatever is present, comparable in this respect to the orange-stained central portion of the mature *Canna* grains already mentioned. Where the outer starch layers are still intact, no additional orange-staining substance appears. This appears to be a further bit of evidence that the inner portion of mature starch grains has undergone some change.

SWELLING AND SOLUTION.

If starch grains are treated with various reagents, they swell and give some very curious and interesting figures. It is a question whether the swelling caused by such reagents as potassium hydroxide, acetic acid, chromic acid, chloral hydrate or hot water is due to imbibition of the reagent between the particles of starch substance or to molecular changes in the particles themselves.

Potato starch grains treated with chromic acid (15%) show at first a slight enlargement of the hilum crack; then the formation of fine radiating cracks, arranged like the barbs of a feather, beginning at the hilum and extending to near the posterior end of the grain. Frequently this area of radiating lines runs part way toward the opposite end of the grain from the hilum and then divides, forming a V-shaped figure. This area spreads and draws toward the margin, and an opening is formed in the central part of the grain. Further, the grain continually increases in size till the outer layers are ruptured. This usually takes place at a point near the hilum. The whole interior of the grain is dissolved out, and the layers at the posterior end and extending part way down the sides are all that remain. The limits of these layers show very sharply, and in some of them fine radial lines appear.

If a potato starch grain is treated with potassium hydroxide (10%), a crack forms, beginning at the hilum and extending toward the posterior end of the grain (Pl. XL, Figs 44-50). Narrow radiating cracks gradually extend from the central crack toward the periphery. The hilum crack enlarges, and at

the same time the layers at the anterior end of the grain begin to push out.

When once this swelling of the anterior part of the grain commences, it proceeds rapidly (Fig. 45). The crack at the hilum continues to enlarge, forming a large cavity in this portion of the grain (Figs. 46, 47). The size of the anterior part of the grain soon considerably exceeds that of the posterior part. When the anterior part of the grain has swollen considerably, a peculiar invagination takes place beginning at the outside at a point near the hilum (Figs 48–50). This infolding appears to relieve the tension caused by the formation of a large internal cavity. The posterior portion of the grain is the last to swell, and if the grain is stained by the triple stain this unswollen portion takes a faint violet color and the swollen portion an orange color. Undoubtedly this swelling of the grains is caused by the absorption of water in the layers. The parts immediately surrounding the hilum take up the water most readily and are the first to swell. The outer layers of the grain are thinnest at the anterior end of the grain, and it is at this end that the stretching is greatest. The crack which begins at the hilum extends through the central part of the grain toward the posterior end and evidently follows the direction of the most readily absorptive material. The layers at the posterior end of the grain are less easily penetrable by the water and maintain their form for a longer time than the inner portions.

Krabbe is of the opinion that the streaming motions which take place when a crystal of alum is brought into contact with a solvent also play an important part in the solution of starch grains by diastase.

More recently, Goldschmidt (12, p. 656) obtained corrosion figures on spheres of calcium carbonate subjected to the action of strong acids, which in their earliest stages show a strong resemblance to those formed in the wheat starch grain as the result of diastase action. The figures which appear in the calcite crystals take the form at first of hemispherical hollows; these Goldschmidt attempts to explain by the supposition of the presence of molecular streams directed toward the

crystal in a line perpendicular to its surface and of returning streams which pass in the opposite direction carrying portions of the crystal in solution.

It seems quite possible to explain the small hemispherical depressions which appear in the early stages of solution by diastase in the same way, as the result of molecular streams between the solid and the solvent. But it is only the figures formed at the beginning of the corrosion of the starch grain that may thus be accounted for. The later stages in corrosion, in which the canals penetrate more deeply into the grain, and which in some cases follow concentric lines, are explained by the fact that certain layers in the interior of the grain are more readily acted upon than others by the diastase solution, and in this way the solution follows the easily soluble layer as the path of least resistance. In the wheat starch grain, it could not be determined which layers of the unstained grain, the highly refractive or the slightly refractive, form the solution paths, as the diastase causes the grain to take a fairly homogeneous stain.

THE DEVELOPMENT OF THE STARCH GRAIN.

Regarding the development of the starch grain, three general views have been held. The first, that the outside of the starch grain is the part first formed, later growth taking place toward the center, was proposed by Munter (22, p. 194) in 1845. He treated the starch grains from the rhizome of *Gloriosa superba* L. with sulphuric acid, and because water appears to be drawn out from the central layers and a large crack is formed in the hilum region, he concluded that the central layers are softer and more watery, and therefore younger, than the outer layers. A similar conclusion was reached by Walpers in 1851 (45, p. 905), in his studies on arrow-root starch. Hartig, in 1855 (14, p. 905), examined the starch of *Canna* and potato and came to a somewhat similar conclusion, that the growth is from the outside toward the center. Nägeli (24), in 1858, proposed the theory of growth by intussusception for all organic structures including the starch grain. This meant to

him that all growth takes place in the interior of the grain, and that it proceeds from the surface inward.

The third view, which is universally accepted at the present day, is that the starch grain grows by the addition of concentric layers. Fritzsche (11), in 1834, noted that the outer layers were more resistant to acids and alkalies than the inner and did not consider that the last layers formed need necessarily be less resistant. Cruger (4), in 1854, made the highly interesting suggestion that a layer of substance between the grain and the plastid is a starch-forming substance. This view is strikingly in harmony with the facts brought out on material carefully fixed and stained by modern cytological methods as described in this paper.

The question of the permanence of the plastid about the entire grain is closely associated with that of its method of growth. Schimper (35, 36), in 1880 and 1881, studied the starch in the cortical parenchyma of the stem of *Philodendron grandifolium* and the medullary parenchyma of *Peperomia stenocarpa* and concludes that these grains are invariably found at first enclosed entirely by plastids; but the material of the plastids is soon broken through and the starch grains project freely into the protoplasm. This conclusion of Schimper's is probably incorrect and due to the fact that on large starch grains the plastid becomes so thin on the anterior portion of the grain as to be visible only with difficulty. In a later paper, Schimper finds that when starch formation is most active the plastid may disappear to an almost invisible remnant and may again regain its former size when starch formation ceases, but he did not change his views as to the ability of the starch grain to project freely into the surrounding protoplasm.

He states that in the large eccentric grains of *Dieffenbachia* the growing end is the one upon which the plastid is fastened, and that when a portion of the starch grain projects freely into the cytoplasm and comes in contact with a second plastid an addition of layers may be made at that point also. In this way he accounts for the peculiar branched grains found so commonly in this plant. It seems quite probable that the

regions in which the plastid is thicker are all parts of one plastid and not separate plastids, as Schimper supposed.

The fact that after a starch grain is partially dissolved in the plastid, leaving an irregular and corroded outline, new layers are added filling up the depressions in the corroded surface, is offered as further evidence by Schimper (36, p. 187) in favor of the theory of external growth. It was found that in the development of the storage starch grains of *Dolichos lablab,* periods of solution were followed by periods of growth of the grain in the plastid. After a period of solution, the outline of the remnant of the starch grain is very irregular, but the new layers added fill up the depressions and form regular layers around the corroded fragment.

Salter describes in considerable detail the development of a potato starch grain. There first appears, when the grain is stained with the triple stain, a rounded white body in the center of the plastid. In this body there soon appears a dark violet dot at the center. A narrow pale violet zone is next seen surrounding the center point. This zone darkens in color at the margin and becomes the first lax lamina, eccentricity being already indicated by the fact that it makes its appearance first on the side turned toward the thicker part of the leucoplast. The second lamina appears in the same manner as did the first, the peripheral portion remaining colorless.

I have examined starch grains from a potato for the stages in growth and find that while the first appearance, that is of the colorless body in the plastid, is as Salter describes, there appears to be no eccentricity of the grain shown when the first violet layer forms, and indeed several violet layers usually form before there is any tendency toward eccentricity (Pl. XXXVIII, Fig. 14). Later in the development, the plastid collects more at one side of the grain and eccentric layers are formed.

In suitable *Canna* material stained by the triple stain of Flemming, we find the different stages in the development of a starch grain very clearly shown (Figs. 2-5). Certain of these show no signs of lamination, others no larger in size show one or two pale violet circles but no broad violet layers (Fig. 3).

Still other grains show a pale violet region at the center (Fig. 9). Slightly larger grains show a central dark violet region, surrounded by a pale violet layer, and this in turn by an orange-stained peripheral layer (Fig. 5). The early violet layers appear to be made up of starch substance distinct from the orange-staining material of which the young grain is composed. As the grain enlarges, more violet layers form and the orange peripheral layer retains a fairly uniform thickness around the grain (Figs. 10-12, 34).

Frequently young grains of *Canna* appear which are stained entirely orange with the exception of one or two minute dots (Fig. 16). These dots are no doubt the beginnings of regions which will later take the violet stain. A grain in a neighboring cell (Fig. 15) is enclosed by a plastid. The grain is stained orange, but with a small violet-stained region at the center which is evidently made up of two parts, each with its own hilum.

A small grain which has an eccentric position in the leucoplast, but which has not as yet any eccentric layers, is often present (Fig. 13). A concentric grain of *Canna* often shows a thin leucoplast, a broad orange layer and a pale violet central region (Fig. 14).

In many large grains the leucoplast can be traced entirely around the periphery (Fig. 33); in others (Fig. 31) but a remnant of it remains.

Certain grains show the effects of solution in the plastid and subsequent growth, with a shifting of the plastid (Fig. 32). The layers in the corroded portion show the effect of corrosion most strongly at the posterior end of that portion of the grain. With a period of renewed growth, the plastid shifts its position and the new layers are put down at an angle of about 45° to the old. Probably the plastid remains as a membrane around the grain, but the layers appear to be deposited only where the plastid is thickest.

In the concentric starch grains found in the seed of *Coix lachryma,* the small grains stain completely orange and show the plastid as a layer of uniform width at the periphery;

slightly larger grains show the central portion of the grain stained violet and the peripheral portion orange.

In the chloroplasts in the central portion of the leaf of *Pellionia daveauana,* several assimilation starch grains are found if the leaf is examined at the close of a bright day (Figs. 21, 22, 24). They vary in number from one to four in a plastid, and quite commonly the plastid is stretched to a thin membrane at certain points. In form, the grains are round, oval or lens-shaped. These grains show no lamination whatever, but a faint crack is present in the middle of the grain. Chloroplasts from leaves of the same plant, if examined the following morning, show that in many cases the starch grains have been entirely removed (Fig. 25); in other cases, slender remnants of the starch grains remain. These remnants show the effects of solution equally on all parts of the surface (Fig. 23).

In all the above cases the grain begins as a more or less strongly orange-stained body, which may well represent a mass of the same transition substance which is found as a peripheral layer in the later stages of growth.

CONCLUSIONS.

A specially differentiated orange-staining layer is present on the periphery of the starch grains from the following plant parts: rhizomes of *Canna* and *Dieffenbachia,* stem of *Pellionia,* tuber of potato, false bulb of *Phajus,* kernels of wheat, barley, rye and corn, and seeds of *Coix.*

In all these cases it is probable that the grains were either growing or being dissolved away at the time the preparations were made. A notable case described above was that of the starch from a rhizome of *Canna,* which had lain dormant through the winter, but from which a vigorous shoot was growing at the time the material was fixed. This starch showed an orange-staining peripheral layer on nearly every grain. In this case, the outer layers of the starch grain were slightly corroded, and the starch was evidently being used for the development of the shoot. Starch from the rhizomes of mature

Canna plants show the peripheral layer equally well, and at this time the grains were probably in a growing condition.

As noted, starch grains from the rhizomes of *Dieffenbachia* and the false bulbs of *Phajus*, which show the peripheral layer, were from plants which were presumably actively storing starch.

The germinating grains of wheat, barley, rye and corn and the seeds of *Coix* show starch grains which have the orange-stained layer at the periphery, and this is clearly a corrosion layer. Thus we find this layer present both in starch grains which are growing and in those which are being used up, and the evidence is strong that it is a transition substance laid down as a continuous layer between the plastid and the starch strata.

In the case of all the above starches, the orange-stained zone is not due simply to the washing in of the orange stain; this is the only region stained by the ordinary exposures to orange, and a much longer exposure does not stain the layers of starch adjacent to this peripheral layer. Further evidence that there is a differentiated peripheral layer is obtained by the careful use of the ordinary iodine staining, using a dilute solution of iodine in water. A peripheral layer remains unstained while the inner layers are colored violet.

The facts show clearly enough that there is a transition layer present between the plastid and the starch grain, and that this layer differs characteristically in its staining reactions from the starch of the inner layers. I am of the opinion also that this difference in staining reaction is evidence that the peripheral layer is chemically different from the layers beneath.

I have, in a preliminary paper (5), advanced the hypothesis that this peripheral layer is a viscid mother substance which becomes more and more concentrated by additions from without until layers of starch are laid down on its inner surface. Where the plastid surrounds the starch grain as a layer uniform in thickness, we may suppose that the material in the peripheral layer is of the same density at every point, this density increasing by the addition of fresh material till a layer

of starch of uniform thickness is crystallized; if, on the other hand, the plastid is thicker on one part of the grain, more material will be added to the peripheral layer at that part, and if we consider the mother substance of this layer to be too viscid to allow the added material to spread readily to the opposite end of the grain, the thicker parts of the layer will be deposited at the part of the grain where the plastid is thickest. All available evidence seems to favor this hypothesis as an explanation of the peripheral layer and the method of growth of the grain.

Although in many cases it is impossible to discover the plastid on the starch grain in thin sections, it is probable that it remains on the grain through the stages of growth and solution. It is exceedingly thin and oftentimes is removed in the preparation of the slide.

Salter claims to have found plastids on all the grains of *Pellionia*, but on the material from the potato he was unable to find the plastid in every case.

By a careful series of observations on the same grain treated successively with different reagents, I have convinced myself that in addition to more or less dense layers of starch, there are sharp lines, as shown in Figure 43, *1* and *2*, which mark the boundaries of highly refractive layers, and also spaces or open water-filled crevices which widen or become more narrow with the swelling and contraction of the adjacent dense layers.

The layers which take the deepest color with gentian violet and iodine are in general the densest layers. They are highly refractive when mounted in water, and stains are removed from them with difficulty. In the case of precipitates formed in the grain, of course the conditions are just the opposite of those in ordinary staining, and, as described above, there is no question that the zones of granules, formed by precipitating methyl violet with calcium nitrate or with picric acid, lie in the more open watery strata and in the crevices between the denser strata of the grain.

Meyer holds that the loose and not the dense layers take the deepest stains, and this view has since been accepted by Sal-

ter. It seems to me natural that the parts of the grain which contain the most solid starch substance would be the ones which are most deeply colored, and the evidence from Figures 42 and 43 is very convincing that this is the case. This is also evidence that the stains are not simply held mechanically between the particles of starch but that they enter into combination with the latter.

In old grains of *Canna* it is often impossible to differentiate layers at the organic center of the grain, the material in this region often appearing as a homogeneous mass. If such grains be crushed so that radial cracks extend through the dense peripheral layers, and if these crushed grains are then stained with gentian violet and orange, it will be found that the entire interior mass of the grain which does not show stratification is also bright orange in color. The staining confirms the observation that the central region of old grains may have been altered characteristically in its nature. In corroded grains, this central portion is often stained orange after the peripheral layers have been penetrated by corrosion channels; or, where corrosion has continued, the central mass may be entirely removed before the peripheral layers show much alteration.

As noted above, if a potato starch grain is treated with a 10 per cent potassium hydroxide solution, the region surrounding the hilum becomes granular in appearance and is the first portion of the grain to swell. The outer starch layers are the last to be affected, showing that they are, no doubt, the most resistant layers of the grain.

From these facts, it seems probable that the central portion of old starch grains has been so modified that it is different in composition from the more peripheral layers. It may be a transition substance similar to that produced by the action of diastase in corroding the grains, as noted further below. Fischer finds that the material from this region can be squeezed out as a fluid mass from grains mounted in water.

As just noted, it was found that where large eccentric grains such as those of the *Canna* or the potato are subjected to the action of diastase, channels are formed passing through the outer dense layers more or less distinctly, but when the inte-

rior of the grain is reached the corrosion is more general, with the result that the interior is dissolved out.

Diastase acts somewhat differently in the grains of wheat and barley; in these grains, channels are often formed running from the periphery to the center. These channels later spread along certain of the concentric layers, probably the less dense, although this could not be definitely determined.

Salter, on the other hand, is of the opinion that, because young starch grains are stained with difficulty by the ordinary stains and take up the orange of the triple stain, they are dense and homogeneous starch masses. For the same reasons, he is of the opinion that the peripheral layer of older starch grains is the densest layer in the grains. His reasons do not appear to be well founded. The orange stain combines readily with the material formed in the corrosion of the starch grain and also, as found by Timberlake, with the substance formed in the manufacture of the cell plate. These are transition substances, and it is but natural to suppose that the orange-staining peripheral layer of the starch grain is a third transition substance. There is small doubt that this layer differs in chemical composition from the violet starch layers, and all the evidence seems to indicate that it is not a dense layer, but rather a loose layer of transitory nature.

BIBLIOGRAPHY.

A fairly complete bibliography up to 1895 is given by Meyer (18).

1. Buetschli, O.: Über den feineren Bau der Stärkekörner. *Verh. Natur.-hist. Med. Verein Heidelberg, N. F.,* v.
2. ——Über die Herstellung künstlicher Stärkekörner oder von Sphaerokrystallen der Stärke. *Verh. Natur.-hist. Med. Verein Heidelberg,* v.—1897.
3. ——: Untersuchungen über Amylose und Amyloseartige Körper. *Verh. Natur.-hist. Med. Verein Heidelberg,* vii.
4. Cruger: Westindische Fragmente, III. *Bot. Zeit.*—1854.
5. Denniston, R. H.: The structure of the starch grain. *Trans. Wis. Acad. Sci., Arts and Letters,* xiv.—1904.
6. Ewart: Assimilatory inhibition. *Journ. Linn. Soc.,* xxxi.—1895.
7. Fischer, H.: Über Inulin, nebst Bemerkungen über den Bau der geschichteten Stärkekörner. *Cohn's Beitr. zur Biol. der Pflanzen,* viii.—1902.
8. ——: Über Stärke und Inulin. *Beih. zum. Bot. Centralbl.,* xii.—1902.
9. ——: Microphotogramme von Inulinsphaeriten and Stärkekörner. *Ber. Deutsch. Bot. Ges.,* xxi.—1903.
10. ——: Über Stärkekörner und ihr Verhalten gegen Farbstoffe. *Beih. zum. Bot. Centralbl.,* xviii.—1905.
11. Fritzsche: Über das Amylum. *Ann. Physik und Chemie.*—1834.
12. Goldschmidt, V.: Zur Mechanik des Lösungsprocess. *Zeitschr. f. Krystallographie und Mineralogie,* xxxviii.—1904.
13. Gruess: Über das Eindringen von Substanzen besonders Diastase in Stärkekörner. *Jahrb. f. wiss. Bot.*—1895.
14. Hartig, T.: Über den Bau des Stärkemehls. *Bot. Zeit.*—1855.
15. Kraemer, H.: On the continuity of protoplasm. *Proc. Amer. Phil. Soc.,* xli.—1902.

16. KRAEMER, H.: The structure of the starch grain. *Bot. Gaz.*—1902.
17. ——: Further observations on the structure of the starch grain. *Bot. Gaz.*—1905.
18. MEYER: Untersuchungen über die Stärkekörner.
19. MIKOSCH: Über die Entstehung der Chlorophyllkörner. *Sitzungsber. Wiener Akad.*—1885.
20. MOHL: Vermischte Schriften.
21. ——: The vegetable cell (Trans. by Henfrey.)
22. MUNTER: Über das Amylum der *Gloriosa superba* L. *Bot. Zeit.*—1845.
23. NAEGELI, C.: The utricular structures in cells. *Roy. Soc. Rept. on Botany.*—1849.
24. ——: Die Stärkekörner.—1858.
25. ——: Das Wachstum der Stärkekörner durch Intussusception. *Bot. Zeit.*—1881.
26. NEWCOMBE, F. C.: Cellulose enzymes. *Ann. of Bot.*—1899.
27. NOLL, F.: Die geformte Proteine im Zellsafte von *Derbesia*. *Ber. Deutsch. Bot. Ges.*, xvii.
28. PURJEWITSCH, K.: Über die Wabenstructur der pflanzlichen organischen Körper. *Ber. Deutsch. Bot. Ges.*, xv.—1897.
29. RODEWALD and KATTEIN: Über natürliche und künstliche Stärkekörner. *Zeitschr. f. Physik. Chemie*, xxxiii.
30. ROTHERT, W.: Einige Bemerkungen zu Arthur Meyer's Untersuchungen über die Stärkekörner. *Ber. Deutsch. Bot. Ges.*—1897.
31. SACHS, J.: Über den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörner. *Bot. Zeit.*—1862.
32. SALTER: Contributions to a fuller knowledge of starch grains.
33. ——: Zur näheren Kenntniss der Stärkekörner. *Jahrb. f. wiss. Bot.*, xxxii.—1898.
34. SCHIMPER, W.: Untersuchungen über die Chlorophyllkörner. *Jahrb. f. wiss. Bot.*—1885.

35. Schimper, W.: Untersuchungen über die Entstehung der Stärkekörner. *Bot. Zeit.*—1880.

36. ——: Untersuchungen über das Wachsthum der Stärkekörner. *Bot. Zeit.*—1881.

37. Schmitz: Die Chromatophoren der Algen. *Verh. naturw. Ver. preuss. Rheinlande und Westfalen*—1883.

38. Strasburger, E.: Über den Bau und das Wachsthum der Zellhäute.

39. Timberlake, H. G.: Starch formation in *Hydrodictyon*. *Ann. Bot.*, xv.

40. ——: Development and function of the cell plate in higher plants. *Bot. Gaz.*, xxx.—1900.

41. Verworn, M.: General physiology (Trans. by Lee).

42. Walpers: Nachtrag zu dem Aufsatze über Arrow-root. *Bot. Zeit.*—1851.

43. Winkler: Untersuchungen über die Stärkebildung in den verschiedenartigen Chromatophoren. *Jahrb. f. wiss. Bot.*, xxxii.—1898.

EXPLANATION OF FIGURES.

All figures (except Figs. 44–50) were drawn with the aid of the Abbé camera lucida.

All figures (except Figs. 17–25, 32) are from the rhizome of *Canna*.

The figures in Plates XXXVIII and XL have a uniform magnification of 875 diameters, unless otherwise mentioned. Figures in Plate XXXIX are magnified 1,220 diameters.

ABBREVIATIONS.

STAINING REACTIONS:

V =very light violet.
V^1=pale violet.
V^2=violet.
V^3=dark violet.
V^4=very dark violet.
O =pale orange.
O^1=orange.
O^2=dark orange.

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l =leucoplast.
c =chloroplast.

PLATE XXXVIII.

EXPLANATION OF PLATE XXXVIII.

Fig. 1. Typical grain from a rhizome of *Canna.*

Figs. 2–5. Series showing development of grain of eccentric form.

Figs. 6–8. Young grains before formation of violet-staining portion.

Fig. 9. Young grains showing violet portion at center.

Figs. 10, 11. Young grains showing formation of eccentric violet-staining layers.

Fig. 12. Eccentric grain in leucoplast.

Fig. 13. Small grain in leucoplast.

Fig. 14. Young grain with leucoplast as thin uniform layer around the periphery.

Fig. 15. Young grain with two hila.

Fig. 16. Young grains with two hila each.

Figs. 17–20. Development of eccentric grain in chloroplast in stem of *Pellionia Daveauana.*

Figs. 21, 22, 24. Chloroplast in leaf of *Pellionia Daveauana,* fixed at the close of a bright day and containing large assimilation starch grains (x 1,750).

Figs. 23, 25. Chloroplasts from same plant, fixed after ten hours in darkness. Fig. 23 shows remnants of starch grains remaining. Fig. 25 shows the plastid completely freed from starch.

Figs. 26–29. Starch grains artificially corroded by diastase.

Fig. 30. Corrosion of grain in the plastid. The anterior end of the grain is reduced to a point and takes the orange stain.

Fig. 31. Compound grain showing orange-stained peripheral layer and portion of leucoplast.

Fig. 32. Grain from *Dieffenbachia seguina* showing the effects of solution and subsequent growth in a new direction, caused by shifting of plastid.

Fig. 33. Large grain showing leucoplast and orange-stained peripheral layer surrounding violet portions of the grain.

Fig. 34. Grain showing broad orange layer.

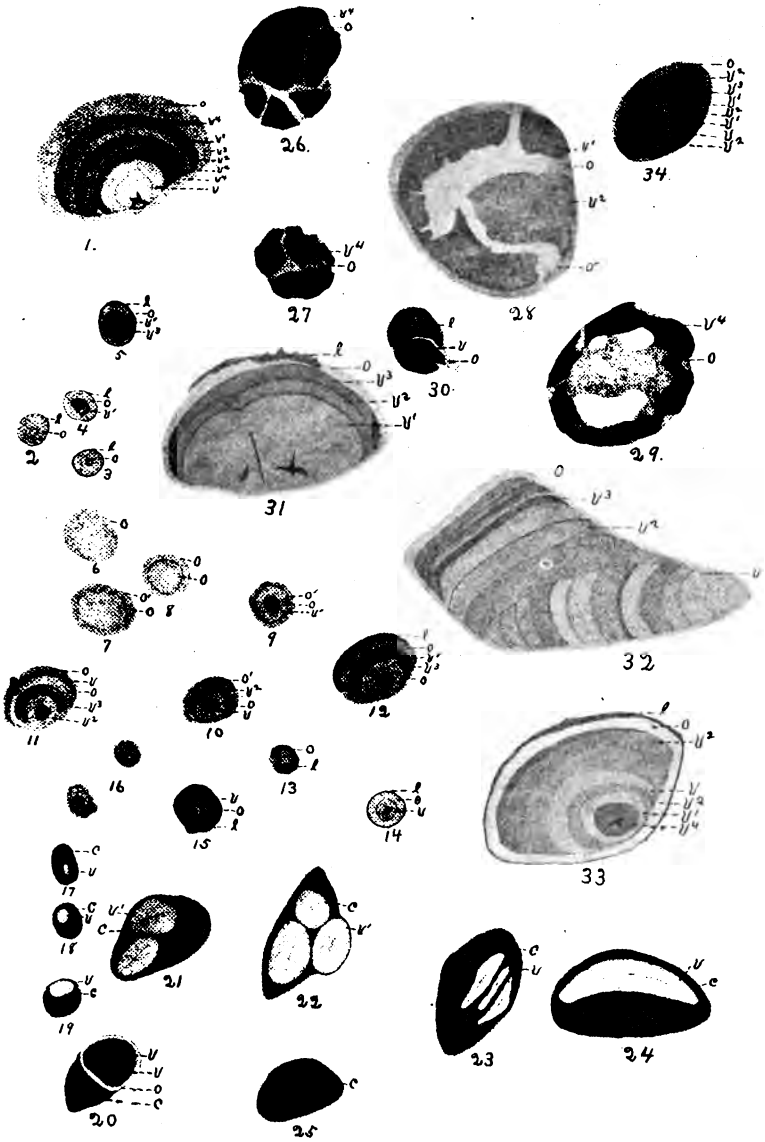

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PLATE XXXIX.

EXPLANATION OF PLATE XXXIX.

Fig. 35. Grain showing orange layer divided at posterior portion of grain by narrow violet layer. Grain surrounded by thin leucoplast.

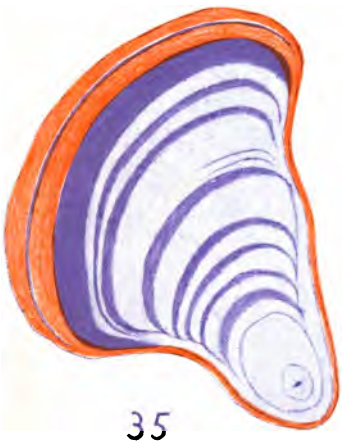
Fig. 36. Outer violet layer completely surrounding violet portions of the grain.

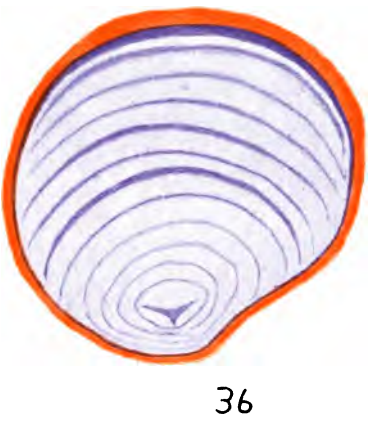
Fig. 37. Grain showing thin leucoplast entirely surrounding grain and broad orange peripheral layer.

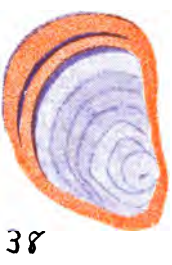
Fig. 38. Grain showing leucoplast as thin layer on periphery; a broad orange layer, separated from a pale orange layer at the posterior end of the grain by a crescent-shaped band of violet-staining starch.

Fig. 39. Grain showing the effect of normal corrosion in the plastid.

Figs. 40, 41. Corroded grains from germinating wheat.







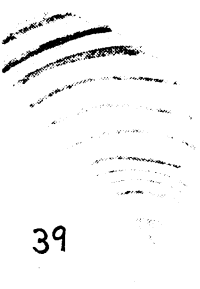


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PLATE XL.

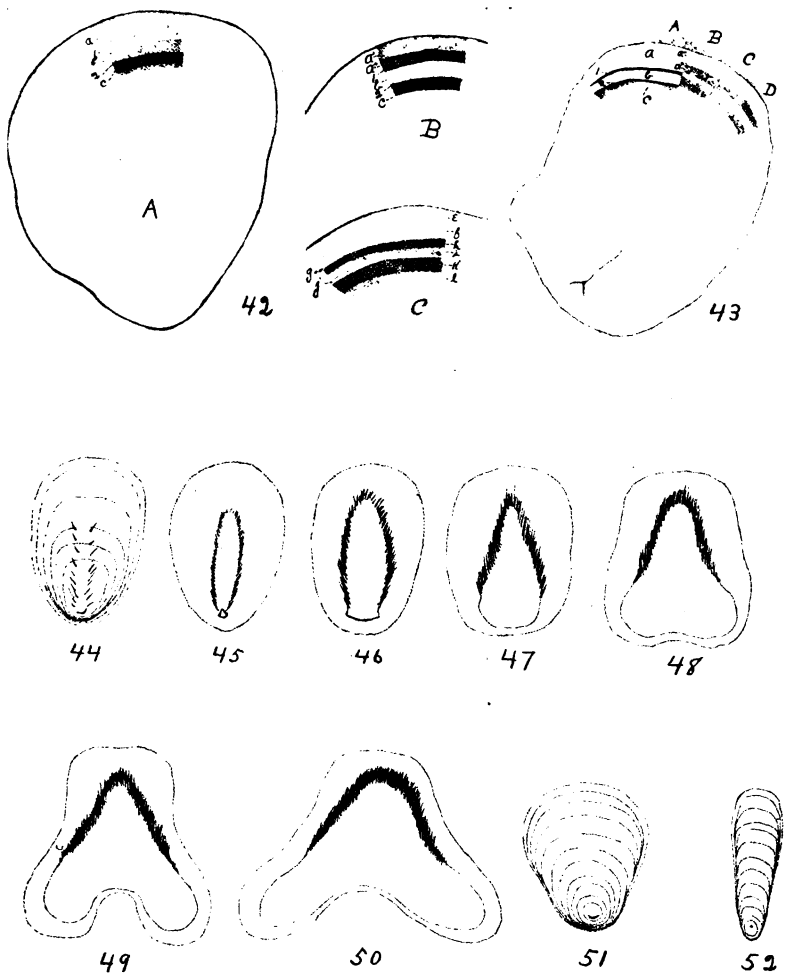
EXPLANATION OF PLATE XL.

Fig. 42. A. Grain mounted in water.
B. Same grain stained with dilute iodine water.
C. Same grain stained with gentian violet and orange G.

Fig. 43. Grain showing appearance of layers when mounted successively in water, iodine water, alcohol and Flemming's triple stain.

Figs. 44–50. *Canna* grain showing successive stages in the action of 10% potassium hydroxide.

Figs. 51, 52. Grain showing surface and edge views.



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